

FLYING FROM ILLUMINATED CULTURES OF
SIBIRIA ASIA

BEH LAM SIEW

FACULTY OF SCIENCE AND TECHNOLOGY
UNIVERSITY COLLEGE OF SCIENCE AND TECHNOLOGY MALAYSIA

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Flavonoids from in vitro cultures of striga Asiatica.



PERPUSTAKAAN
KOLEJ UNIVERSITI SAINS & TEKNOLOGI MALAYSIA
21030 KUALA TERENGGANU

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FLAVONOIDS FROM *IN VITRO* CULTURES OF *STRIGA ASIATICA*.

By

Beh Lai Siew

**Research Report submitted in partial fulfillment of
the requirements for the degree of
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PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: **Flavonoids from *in vitro* cultures of *Striga asiatica*** oleh Beh Lai Siew, No. Matrik: UK 7493 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Kimia sebagai memenuhi sebahagian daripada keperluan memperolehi **Ijazah Sarjana Muda Sains (Sains Kimia)**, Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:


.....

Penyelia Utama

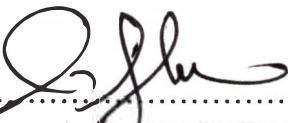
Nama: **Dr. Habsah binti Mohamad**

Dr. Habsah Mohamad

Cop Rasmi:

Pensyarah
Jabatan Sains Kimia
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu.

Tarikh: 9/5/05
.....



.....

Penyelia Kedua (jika ada) **DR. AZIZ BIN AHMAD (Ph.D)**
LECTURER

Nama: **Dr. Aziz bin Ahmad**
Dept of Biological Sciences
Fakulti of Science and Technology
University Collage of Science
and Technology Malaysia
21030 Kuala Terengganu.

Cop Rasmi

Tarikh: 12/5/2005
.....


.....

Ketua Jabatan Sains Kimia

Nama: **Prof. Madya Dr. Ku Halim Ku Bulat**

Cop Rasmi:

PROF. MADYA DR. KU HALIM KU BULAT
Ketua
Jabatan Sains Kimia
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu.
Tel: 09-6583257

Tarikh: 9th May 2005
.....

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LIST OF ABBREVIATIONS

| | |
|----------------------------|--|
| λ | Wavelength |
| μM | Micromolar |
| A | Absorbance |
| ACC | 1-aminocyclopropane-1-carboxylate |
| ATR | Attenuated Total Reflectance |
| BAP | 6-Benzylaminopurine |
| c | concentration of the species under consideration (mol/L) |
| CD_3COCD_3 | Acetone- d_6 |
| CHCl_3 | Chloroform |
| CO_2 | Carbon dioxide |
| D_2O | Deuteriumoxide |
| DMSO-d_6 | Deuteriated Dimethyl Sulfoxide |
| DPPH | 1,1-diphenyl-2-picrylhydrazol |
| EIMS | Electron Impact Mass Spectrometry |
| eV | electron-Volt |
| Fe^{2+} | ion Ferum (II) |
| g/L | gram per liter |
| H_2O | Water |
| IR | Infra Red |
| KBr | Potassium Bromide |

| | |
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| L | path length through the sample |
| MeOH | Methanol |
| mg | Milligram |
| MS | Murashige and Skoog |
| NaCl | Sodium Chloride |
| BuOH-EtOAc | Butanol-ethylacetate |
| NMR | Nuclear Magnetic Resonance |
| O ₂ | Oxygen |
| °C | Degree Centigrade |
| % | Percentage |
| p.s.i | Pounds per square inch |
| R _f | Retention factor |
| STD | Standard (Mixture of Stigmasterol and β -sitosterol) |
| TLC | Thin Layer Chromatography |
| UV | Ultraviolet |
| ϵ | The coefficient of the material |
| μ g | Microgram |

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ABSTRACT

An experiment was conducted to identify the secondary metabolites from *in vitro* cultures of *Striga asiatica*. The cultures were proliferated in solid and liquid media for six months. The dried sample was extracted in methanol for a week. The methanol extract was partitioned by using n-BuOH-EtOAc (1:1) and H₂O (1:1) followed by MeOH : n-Hexane (1:1) to yield a methanol concentrate, MeOHSGA. Then, it was separated by thin layer chromatography (TLC) and column chromatography yielding two hundred and thirteen fractions. The fractions F7, F10 and F20 were subjected to repeated column chromatography. From fraction F7, the compound of SA1 was purified by recrystallization using chloroform-methanol. In co-TLC, it showed a spot with similar R_f value with the standard (mixture of Stigmasterol and β -sitosterol). SA1 (0.0673 g) posed a melting point at 140-141°C which was similar to β -sitosterol. By Infrared (IR) analysis and Electron Impact Mass Spectrometry (EIMS), SA1 was further confirmed as β -sitosterol. In this experiment, no pure flavonoids compound was obtained. However, the preliminary chemical test showed that flavonoids existed in the sample. The presence of flavonoids in fractions 10C14, S183, F34, S202 and F38, that showed a positive reaction to ferric chloride and DPPH free radical scavenging assay have been detected. The characters of flavonoids in these fractions were analyzed using UV and IR spectrum.

FLAVONOID DARI *Striga asiatica* YANG DIKUTUR DALAM TIUB

ABSTRAK

Suatu eksperimen telah dijalankan untuk mengenalpasti metabolit sekunder dari kultur *Striga asiatica* dalam tiub. Kultur ini dipropagasi dalam media pepejal dan cecair selama enam bulan. Sampel kering diekstrak dengan menggunakan metanol selama seminggu. Ekstrak metanol dipisahkan oleh n-BuOH-EtOAc (1:1) dan H₂O (1:1), diikuti dengan MeOH: n-Hexane (1:1) untuk menghasilkan ekstrak pekat metanol, MeOHSGA. Kemudian, ia dipisahkan dengan kromatografi turus untuk menghasilkan dua ratus tiga belas fraksi. Fraksi gabung F7, F10 dan F20 dipisahkan sekali lagi menggunakan kromatografi turus. Dari fraksi F7, komponen SA1 ditulenkan melalui teknik penghabluran semula yang menggunakan kloroform-metanol. Dari co-TLC, ia mempamerkan satu titik yang mempunyai nilai R_f yang sama dengan piawai (campuran Stigmasterol dan β -sitosterol). SA1 (0.0673 g) menunjukkan takat lebur pada 140-141°C yang sama dengan dan β -sitosterol. Melalui analisis spektroskopi infra merah (IR) dan Spektrometer Jisim Hentaman Elektron (EIMS), SA1 dibuktikan sebagai dan β -sitosterol. Dari eksperimen ini, tiada komponen flavonoid tulen yang diperoleh. Bagaimanapun, ujian kimia awal menunjukkan bahawa flavonoid hadir dalam sample. Kehadiran flavonoid dalam fraksi-fraksi 10C14, S183, F34, S202 and F38 yang menunjukkan reaksi positif kepada ferik klorida dan pengujian pengesan radikal bebas DPPH telah dikenalpastikan. Sifat flavonoid dalam fraksi-fraksi ini dianalisa oleh spektra ultra lembayung (UV) dan IR.